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Seizure control by ketogenic diet-associated medium chain fatty acids

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ABSTRACT

The medium chain triglyceride (MCT) ketogenic diet is used extensively for treating refractory childhood epilepsy. This diet increases the plasma levels of medium straight chain fatty acids. A role for these and related fatty acids in seizure control has not been established. We compared the potency of an established epilepsy treatment, Valproate (VPA), with a range of MCT diet-associated fatty acids (and related branched compounds), using *in vitro* seizure and *in vivo* epilepsy models, and assessed side effect potential *in vitro* for one aspect of teratogenicity, for liver toxicology and *in vivo* for sedation, and for a neuroprotective effect. We identify specific medium chain fatty acids (both prescribed in the MCT diet, and related compounds branched on the fourth carbon) that provide significantly enhanced *in vitro* seizure control compared to VPA. The activity of these compounds on seizure control is independent of histone deacetylase inhibitory activity (associated with the teratogenicity of VPA), and does not correlate with liver cell toxicity. *In vivo*, these compounds were more potent in epilepsy control (perforant pathway stimulation induced status epilepticus), showed less sedation and enhanced neuroprotection compared to VPA. Our data therefore implicates medium chain fatty acids in the mechanism of the MCT ketogenic diet, and highlights a related new family of compounds that are more potent than VPA in seizure control with a reduced potential for side effects.

This article is part of the Special Issue entitled 'New Targets and Approaches to the Treatment of Epilepsy'.

1. Introduction

The medium chain triglyceride (MCT) ketogenic diet has provided one of the most effective therapeutic approaches for children with drug resistant epilepsy (Kossoff et al., 2009; Liu, 2008; Neal et al., 2009; Rho and Stafstrom, 2012; Sills et al., 1986b; Vining et al., 1998). However, its use has been limited by poor tolerability, especially in adults, raising the need for the development of novel therapies based upon this diet. The MCT diet causes a rise in ketone body formation, but this correlates poorly with seizure control (Likhodii et al., 2000; Thavendiranathan et al., 2000). It also causes accumulation of medium chain fatty acids in blood plasma (in particular octanoic and decanoic acids, Fig. 1A) (Haidukewych et al., 1982; Newport et al., 1979; Sills et al., 1986a), although the role of these fatty acids, if any, in seizure control remains unclear.

The short chain fatty acid valproic acid (VPA, 2-propylpentanoic acid), is a commonly used broad-spectrum antiepileptic drug, but is sub-optimal due to numerous side effects: The two most significant of these are teratogenicity (Jentink et al., 2010; Koren et al., 2006), which has been correlated with inhibition of histone deacetylase activity (Gottlicher et al., 2001; Gurvich et al., 2004; Phiel et al., 2001) although other mechanism may also function here; and hepatotoxicity (Lagace et al., 2005; Stephens and Levy, 1992), potentially due to effects on β-oxidation (Elphick et al., 2011; Silva et al., 2008). Additionally, fatty acids with structures related to VPA have also been associated with significant sedative properties, often preventing translation into clinical trials (Bojic et al., 1996; Keane et al., 1983; Palaty and Abbott, 1995). These effects have influenced the search for novel fatty acid structures with increased potency against seizures and with a better side-effect profile than VPA.

In the search for new seizure control treatments, a recent study suggested that the action of VPA involves modification of phosphoinositol turnover in the social amoeba *Dictyostelium discoideum* (Chang et al., 2011). Based on this mechanism, a group of medium chain fatty acids including both MCT-diet associated compounds

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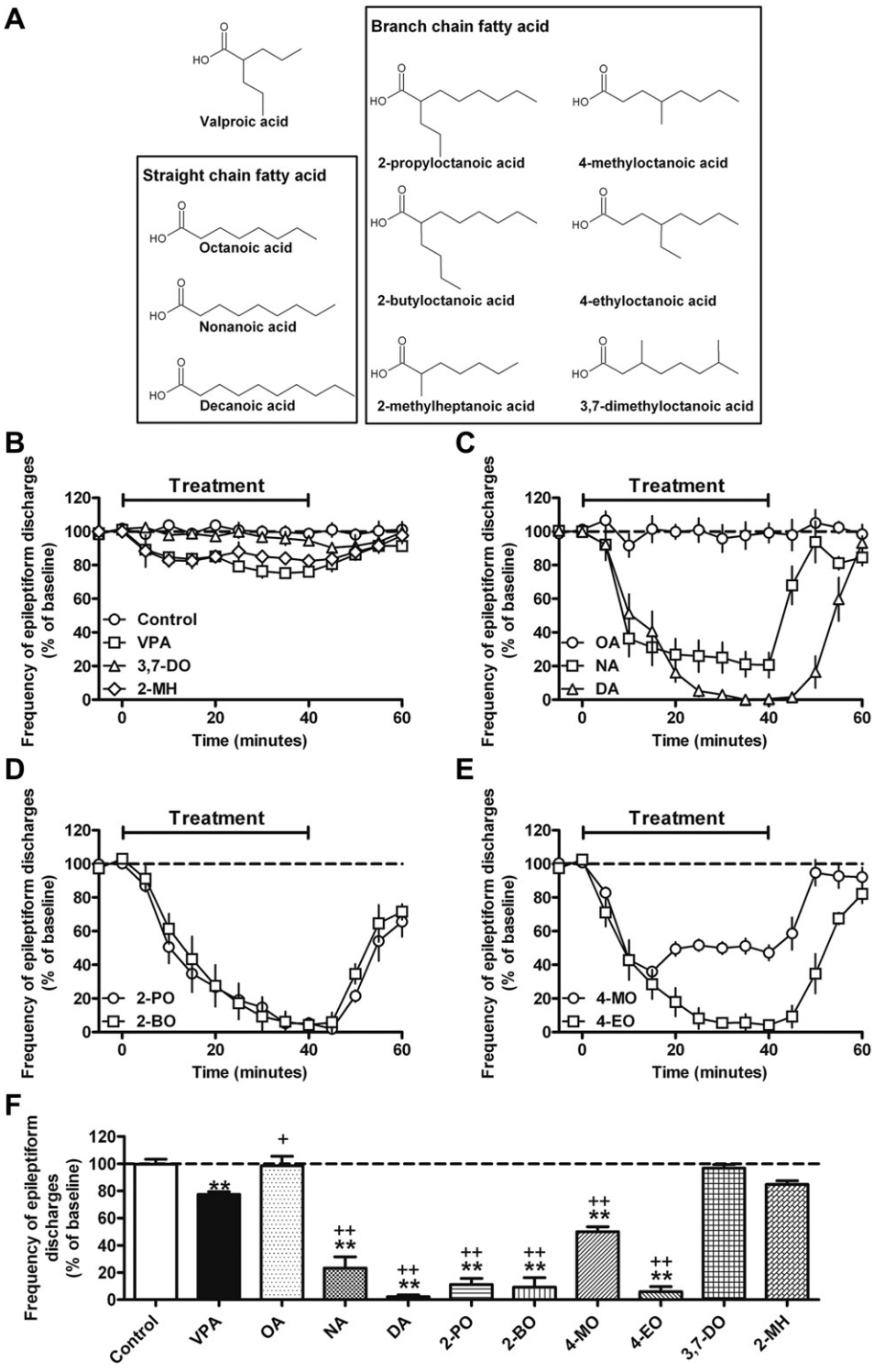

Fig. 1. Structurally-specific medium chain fatty acids strongly reduce frequency of *in vitro* epileptiform activity. (A) A range of medium chain fatty acids were analysed in this study: Straight medium chain fatty acids octanoic (OA), nonanoic (NA) and decanoic (DA) acids contain 8, 9 and 10 carbon backbones respectively. Related structures analysed are derivates of octanoic acid branched at the second carbon (2-propyloctanoic acid (2-PO) and 2-butyloctanoic acid (2-BO)) and the fourth carbon (4-methyloctanoic acid (4-MO) and 4-ethyloctanoic acid (4-EO)); and two related structures, a heptanoic acid derivative (7 carbon backbone) branched on the second carbon (2-methylheptanoic acid (2-MH)), and an octanoic acid derivative branched on both the third and the seventh carbon (3,7-dimethyloctanoic acid (3,7-DO)). The frequency of epileptiform activity is plotted against time following (B) control (DMSO) and 3,7-DO ($n = 3$) gave no effect on epileptiform activity, whereas VPA and 2-MH ($n = 3$) treatment showed a weak effect. (C) The straight chain fatty acid OA showed no effect, whereas a strong effect was shown for NA and DA. (D) Octanoic acid derivatives branched on the second carbon, 2-PO, 2-BO; and (E) on the fourth carbon 4-MO, 4-EO are also highly active. (F) Comparison of the mean frequency of PTZ-induced burst discharges, averaged from 20 to 40 min post compound addition (data shown as means ± SEM). * and ** indicate a significant difference at $p < 0.05$ or $p < 0.01$ compared to control respectively; + and ++ indicate similar levels of significance compared to VPA. Data is provided for all compounds tested at 1 mM from at least five repeats unless indicated. Illustrative trace recordings plotted against time for all compounds are provided in Supplementary Fig. 1.